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Septic shock caused by *Capnocytophaga canis* after a cat scratch

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ABSTRACT

Capnocytophaga canis is an uncommon cause of septic shock. Only three cases have been previously reported in the literature. In this article, we describe the case of a 70-year-old male admitted to the intensive care unit for septic shock of unknown origin. On day 2, one anaerobic bottle out of the two sets taken at admission turned positive with Gram-negative bacilli. The pathogen was identified by 16S rRNA gene as *C. canis*. The strain was characterized and compared to other clinical isolates of *Capnocytophaga* spp.

Key words: asplenia, sepsis, capsule, *Capnocytophaga*, whole-genome sequencing, zoonosis

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26 **Funding:** No external funding was received for this study.

27 **Conflict of interest:** the authors have nothing to disclose. Francesco Renzi is a Research Associate of
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29 **Consent to participate:** NA

30 **Consent for publication:** written consent was given by the patient for anonymous publication of
31 clinical history and laboratory data relevant for the case presentation and publication.

32 **Availability of data and material:** NA

33 **Code availability:** NA

34 **Authors' contributions:** VD contributed with the original idea, case description and wrote to the final
35 version of the manuscripts with the help of MB, VL, FR, GR, AC, and JS. FR performed the
36 characterization of the serovar. GR and AC performed the bacteriological and molecular analyses.
37 MG, NG and VL performed the genomic analysis.

38

CASE REPORT

A 70-year-old male presented with complaints of malaise, fever with chills, and shortness of breath evolving for 6 hours as well as a worsened lumbar pain. His medical history was relevant for chronic lumbar pain, permanent atrial fibrillation and chronic alcohol consumption. He had several episodes of acute complicated pancreatitis in the past. At the age of 57, he underwent a partial pancreatectomy with splenectomy for a complicated pancreatic pseudocyst. His vaccination status was not up to date.

Vital signs on admission were as follows: blood pressure 90/60 mmHg, pulse rate 130 bpm irregular, respiratory rate 15/min, percutaneous oxygen saturation 94%, temperature 40.2°C. The clinical examination indicated a mottled skin and diffuse lumbar pain without focal pain or neurological defect. Laboratory test results revealed a mild leucopenia ($3.7 \times 10^9/L$) with 15% band forms, a mildly elevated C-reactive protein (20.4 mg/mL) and high procalcitonin level ($>100 \mu g/L$). Coagulation tests showed a decreased prothrombin time (29%), prolonged partial thromboplastin time (67%) and low fibrinogen (1.8 g/L) with preserved factor V activity (100%). The patient received a single empirical dose of 2 g of ceftriaxone in the prehospital setting due to the fever and his asplenic condition. Shortly after his arrival in the emergency department, blood cultures were drawn and empirical treatment with piperacillin-tazobactam for septic shock of unknown origin was administered. A computed thoraco-abdominal tomography scan performed that same day showed a left periureteral infiltration without signs of renal obstruction, and some enlarged mesenteric lymph nodes. There was no sign of spondylodiscitis.

Clinical course

Despite volume resuscitation, noradrenaline initiation and antibiotics, the patient rapidly developed a severe vasoplegic shock and was transferred to the intensive care unit (ICU). On day 1, he showed a severely depressed cardiac index ($<1.84 \text{ L/min/m}^2$) despite optimal volume resuscitation. Septic cardiomyopathy was suspected and dobutamine was initiated. The clinical course was associated with acute kidney injury and ICU delirium.

On day 2, a dark purple spot was detected on his left forearm, which was reported by his spouse to be a 4-day old cat scratch. Swelling was absent and the lesion showed no signs of cellulitis, nor lymphadenopathy. On day 2, vasopressor requirements decreased, and shock resolved on day 5. Laboratory findings evolved towards a leukemoid reaction with the highest count of white blood cells on day 4. The patient also developed severe thrombocytopenia with a nadir on day 4 (**Figure 1**). Acute

kidney injury, delirium and coagulation abnormalities resolved with supportive measures. Patient was discharged from ICU on day 8 and he was discharged for rehabilitation on day 26.

Microbiology

On day 2, Gram-negative bacilli grew in one anaerobic bottle out of the two sets taken at admission. No other blood culture turned positive. The morphology of the bacillus was suggestive of a *Capnocytophaga* spp.

Peptide mass fingerprintings of bacterial isolates grown overnight on Columbia Agar with 5% Sheep Blood were determined by matrix-assisted desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS Compass, Bruker Daltonics, Bremen, Germany) according to the manufacturer's instructions. A spectrum was obtained but without any matching organism according to the reference database.

Sequence analysis of PCR-amplified 16S rRNA gene of the isolated strain (GE-031) showed 99.2% (878/885 nt) identity with the type strain (CcD38) of *Capnocytophaga canis*, a species that is not represented in the MALDI Biotyper reference (8431 MSP) library. The isolated strain was tested by PCR for the presence of the *Capnocytophaga canimorsus* capsular types A, B, C, D and E and the results showed that it did not belong to any of the tested serovars. Strain GE-031 appeared genetically close (**Supplementary Methods**) to sequenced *C. canis* strains [average nucleotide identity (ANI) 98.053–98.227] but distant from other *Capnocytophaga* species (ANI <78) (**Supplementary Table**). A core genome single-nucleotide polymorphism (cgSNP) analysis confirmed close phylogenetic relationships between GE-031 and sequenced *C. canis* strains (**Figure 2**).

DISCUSSION

C. canis is a newly described species in the genus *Capnocytophaga* [1]. These Gram-negative bacilli belong to the oral microbiota of dogs and cats [2]. Their pathogenicity for humans was originally hypothesized to be very low [3] because they had not been isolated from human infections. Since the description of the species in 2016, some cases of human infections by *C. canis* have been reported, and several strains isolated from infected humans initially considered as *C. canimorsus* were reclassified as *C. canis* [2]. Suzuki et al characterized 3 strains of *C. canis* isolated from septic patients in Japan [4]. One of the 3 strains reported by Suzuki was also published in a recent case report describing

the first case of septic shock by *C. canis* occurring in an asplenic patient three days after a cat scratch [5].

To our knowledge, our case is the second documented case of septic shock caused by *C. canis* after that described by Taki et al [5]. These two cases share some common clinical characteristics such as a severe septic shock, some features of disseminated intravascular coagulation, leukemoid reaction and a difficult to identify Gram-negative bacillus.

The characterization of our strain showed that it does not belong to the A-E capsular serovars which were found in *C. canimorsus* strains isolated from human infections [6].

The strain GE-031 as well as the three recently characterized *C. canis* strains could not be identified by MALDI-TOF MS because of the lack of *C. canis* reference spectrum in the MALDI Biotyper reference library. In contrast, strain CCUG 17663, considered as *C. canis* on the basis of 16S rRNA gene phylogeny [2], was identified by MALDI-TOF MS as *C. canimorsus* (**Supplementary Table**). However, this strain is closer to *C. canimorsus* than to *C. canis* in terms of (i) cgSNP-based phylogeny (**Figure 1**), (ii) ANI (97.6 and 76.1 for type strains of *C. canimorsus* and *C. canis*, respectively) and (iii) capsular serotype B shared with *C. canimorsus* [2]. Comparison of 16S rRNA gene sequence of strain CCUG 17663 obtained by amplicon sequencing [2] to that reconstructed from the WGS data (this study) revealed 17 single mismatches over the length of 737 aligned nucleotides. All but one of these nucleotide variants in the amplicon sequence corresponded to nucleotides found in *C. canis* strains, suggesting that the chromosomal DNA of CCUG 17663 used in the PCR was contaminated with DNA from a *C. canis* strain.

Our results confirm the advantage of WGS over other molecular and phenotypic assays for delineation of *Capnocytophaga* species and highlight its pathogenic potential in asplenic patients.

123 Figure legends

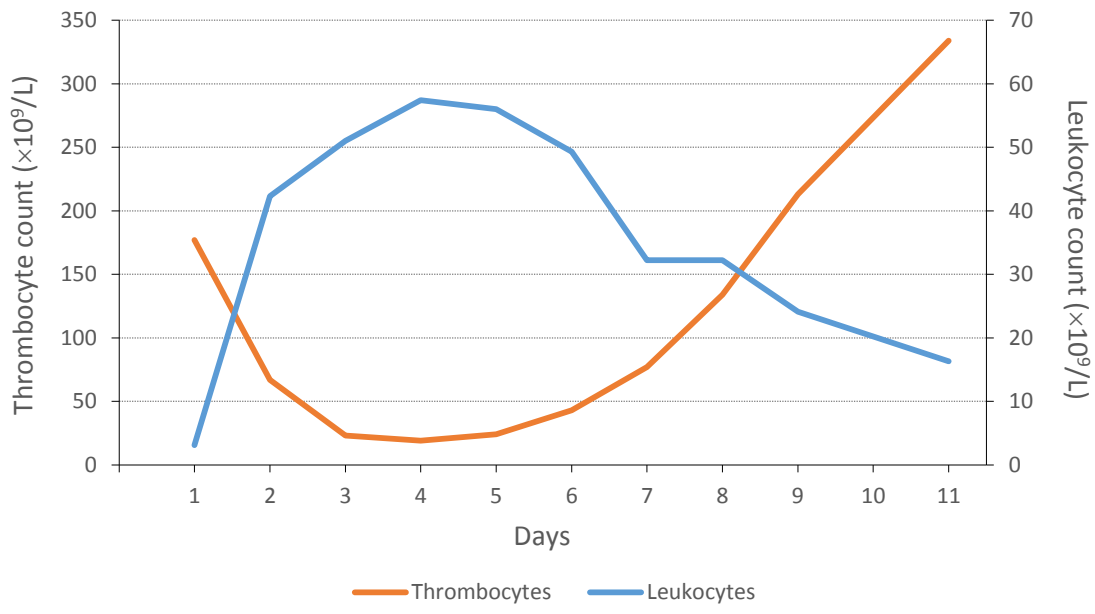
124 **Figure 1. Kinetics of leucocyte and thrombocyte counts during the acute course of infection.**

125 **Figure 2. Relatedness between *C. canis* GE-031 and selected isolates of *Capnocytophaga* genus**
126 **assessed by cgSNP.** The analysis included genomic sequences of *C. canis* isolates available in the NCBI
127 assembly database, type strains (T) of *C. cynodegmi*, *C. canimorsus*, *C. sputigena* and *C. ochracea*, as
128 well as four strains from the CCUG (Culture Collection University of Gothenburg) collection, recently
129 classified as *C. canis* and sequenced for the purpose of this study. Neighbor-joining tree based on
130 comparison of nucleotide sequences of 35,961 cgSNP shared by all analyzed strains was created in
131 SeqSphere+.

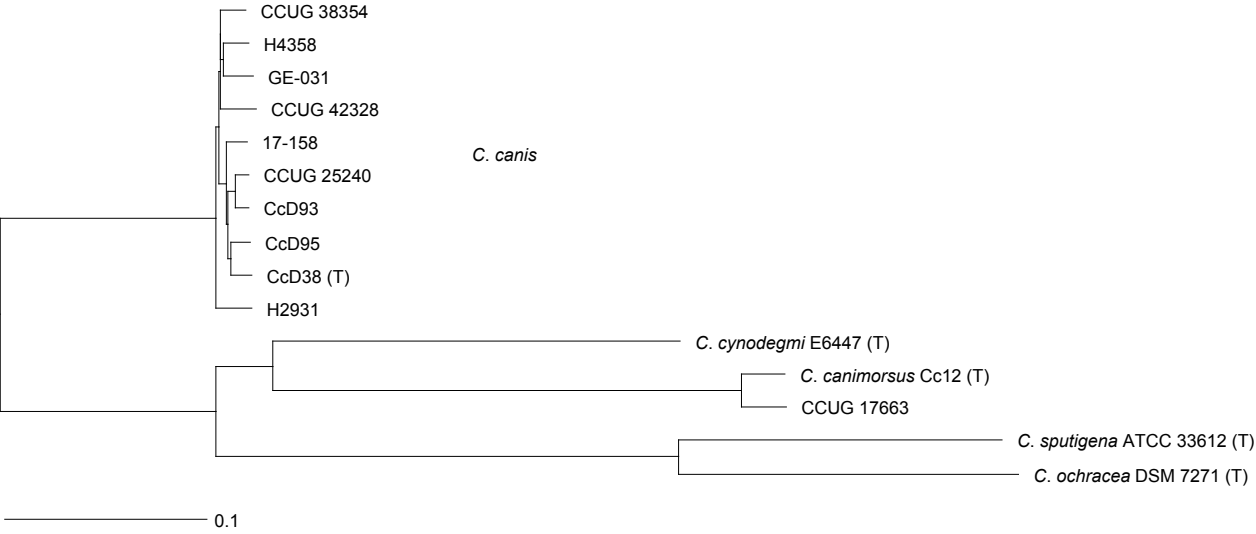
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Figure_1



Figure_2



Supplementary Methods

Genomic analysis

Capnocytophaga strains and growth conditions. The *C. canis* strain GE-031, isolated at the Geneva University Hospitals in this study and the four Swedish *Capnocytophaga* isolates CCUG 17663, CCUG 25240, CCUG 38354 and CCUG 42328 [1], recently described as *C. canis*, were grown on bioMérieux Columbia agar plates for 3 days at 37 °C in CO₂-enriched (5%) atmosphere.

DNA extraction. Several freshly grown bacterial colonies were harvested, transferred into 500 µL of GT buffer (RBS Bioscience, New Taipei City, Taiwan) and shaken in a Nucleospin Bead Tube (Machery-Nagel, Düren, Germany) for 20 min at maximum speed on a Vortex-Genie 2 with a horizontal tube holder (Scientific Industries, Bohemia, NY, USA). After addition of 1 µL of 50 mg/mL RNaseA (Roche), samples were incubated at room temperature for 5 min and centrifuged for 2 min at 11,000 × g. DNA was extracted from 400 µL of the supernatant using the MagCore Genomic DNA Tissue kit on a MagCore HF16 instrument (RBC Bioscience) and eluted in 100 µL of 10 mM Tris-HCl pH 8.

Genomic DNA sequencing. A sequencing library of strain GE-031 genomic DNA, prepared using a Nextera XT DNA Library Prep Kit (Illumina, San Diego, CA, USA), was sequenced (2×250 cycles) on an MiSeq instrument using MiSeq Reagent Nano Kit v2 (Illumina) at Fasteris (Plan-les-Ouates, Switzerland). DNA of other strains was prepared for sequencing with a Nextera DNA Flex Sample Preparation Kit (Illumina) and sequenced in-house on an Illumina iSeq 100 System (Illumina, San Diego, CA, USA) with 151-bp paired-end reads.

Bioinformatics analysis. The Trimmomatic package v.0.36 [2] was used to: (i) remove bases that corresponded to the standard Illumina adapters, (ii) cut any base at the start or the end of a read with a threshold quality below 5 (strain GE-031), (iii) trim low-quality ends of reads at the beginning of a 4-base-wide sliding window with an average Phred quality <5 and a 20-base (strain GE-031) or a 10-base (other strains) wide sliding window with an average Phred quality <30; (v) remove reads that were <90 bases (strain GE-031) or <150 (other strains) in length after trimming. Putative artifactual replicate reads were filtered out using a homemade script that retains the longest sequence among those with identical first 90–100 (GE-031) or 100 (other strains) bases, in either the forward or reverse reads. Any forward or reverse reads without its corresponding paired read were discarded.

To control for DNA contamination, we first removed all reads assigned to the phylum Chordata (to which the species *Homo sapiens* belongs) based on the CLARK [11] v.1.2.5 classification at the phylum level with the default parameters against human genome sequence (GRCh38.p7) [3,4]. Then, we classified the remaining reads using CLARK (with parameters -m 0 -c 0.8) against reference and representative prokaryotic genomes from the NCBI/RefSeq database (downloaded on 14 June 2018) [13] as well as against the CLARK fungal and viral database (downloaded on 14 June 2018). The reads assigned to the class Flavobacteria (to which *Capnocytophaga* belongs) and those that remained unclassified were retained and assembled with SPAdes 3.11.1 [5] (-k 21,33,55,77,99,127 --careful) followed by QUAST v.5.0.2 [15] evaluation.

A *de novo* core genome scheme was created in Ridom SeqSphere+ v.5.1.0 with default settings. The genomic sequence of the *C. canis* strain H2931 was used as a seed. It was aligned with genomic sequences of six other *C. canis* strains available in the NCBI assembly database, type strains of *C. cynodegmi*, *C. canimorsus*, *C. sputigena* and *C. ochracea*, as well as four strains from the CCUG collection (CCUG 38354, CCUG 42328, CCUG 25240, CCUG 17663) recently described as *C. canis* [1] and sequenced for the purpose of this study. Core-genome single-nucleotide polymorphism (cgSNP) analysis was performed on a multiple sequence alignment of 145 concatenated core genes using at least 70% nucleotide sequence identity and 90% alignment coverage. cgSNPs were called exclusively in positions shared by all samples. Potentially indel-related cgSNPs were removed.

Average nucleotide identity (ANI) between the strain GE-031 draft genome assembly and selected *Capnocytophaga* isolates was calculated using pyani [6] with BLAST [7] method.

Data availability. WGS assemblies of *Capnocytophaga* isolates reported in this manuscript have been deposited in NCBI WGS database (BioProject PRJNA578526) under the accession numbers indicated in Supplementary Table 1.

Supplementary Table 1. *Capnocytophaga* strains compared in this study.

Strain	Source of isolation	Country (year) of isolation	ANI with GE-031	Reference(s)	GenBank assembly accession	MALDI-TOF result*
<i>Capnocytophaga canis</i> GE-031	Human blood, after cat bite	Switzerland (2018)			WLVJ000000000 (This work)	Non-typable, <i>Helicobacter canis</i> 1.46
<i>C. canis</i> H2931	Human blood after animal bite	USA (2004)	98.227	Villarma et al (2017) [8]	GCA_002302535.1	
<i>C. canis</i> H4358	Human blood after animal bite	USA (2007)	98.053	Villarma et al (2017) [8]	GCF_002302515.1	
<i>C. canis</i> CCUG 38354 (G36)	Human blood (after cat bite)	Sweden (1997)	98.088	Renzi et al (2018) [1]	WLVJ000000000 (This work)	Non-typable, <i>Clostridium cochlearium</i> 1.44
<i>C. canis</i> CCUG 42328 (G77)	Human blood	Sweden (1999)	98.192	Renzi et al (2018) [1]	WLVH000000000 (This work)	Non-typable, <i>Clostridium difficile</i> 1.32, <i>Hydrogenophaga flava</i> 1.32
<i>C. canis</i> CcD95	Dog mouth	Switzerland (2008)	98.092	Mally et al (2009) [9]; Manfredi et al (2015) [10]; Renzi et al (2015) [11]	GCF_000827695.1	
<i>C. canis</i> CcD38	Dog mouth	Switzerland (2008)	98.123	Mally et al (2009) [9]; Manfredi et al (2015) [10]; Renzi et al (2015) [11]	GCF_000827555.1	
<i>C. canis</i> CcD93	Dog mouth	Switzerland (2008)	98.095	Mally et al (2009) [9]; Manfredi et al (2015) [10]; Renzi et al (2015) [11]	GCF_000827715.1	
<i>C. canis</i> CCUG 25240 (G17)	Human blood, after dog bite	Sweden (1989)	98.182	Renzi et al (2018) [1]	WLVG000000000 (This work)	Non-typable, <i>Bacillus weihenstephanensis</i> 1.26
<i>C. canis</i> 17-158	Human blood	USA (2017)	98.135		GCF_003585975.1	
<i>Capnocytophaga cynodegmi</i> E6447	Mouth of a dog	USA (1979)	77.055	Brenner et al (1989) [12]	GCF_000379185.1	
<i>Capnocytophaga canimorsus</i> Cc12	Blood after dog bite	USA (1961)	75.906	Brenner et al (1989) [12]; Manfredi et al (2015) [13]	GCF_000827635.1	
<i>Capnocytophaga sputigena</i> ATCC 33612	Periodontal lesions	Unknown (1978)	70.720	Leadbetter et al (1979) [14]	GCA_000173675.1	
<i>Capnocytophaga ochracea</i> DSM 7271	Human oral cavity	Unknown (before 07.10.1992)	70.463	Leadbetter et al (1979) [14]; Mavrommatis et al (2009) [15]	GCF_000023285.1	
<i>C. canimorsus</i> CCUG 17663 (G05)	Human aorta	Sweden (1985)	76.179	Renzi et al (2018) [1]	WLVF000000000 (This work)	<i>C. canimorsus</i> 2.2

* The MALDI-TOF identification score values ≥ 2.0 were considered positive. Non-typable = scores < 1.5 , which did not allow accurate strain identification

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